

In vitro activity of Triazolo[1,5-a] pyrimidine carboxylic acid extracted from microalgae *Hapalosiphon welweschii* against the protoscolices of hydatid cyst

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Abstract :

The aim of the present study is testing the activity of Triazolo[1,5-a] pyrimidine carboxylic acid extracted from *Hapalosiphon welweschii* against the hydatid cyst of *Echinococcus granulosus*. . the results was found that the compound of the methanol extract of *Hapalosiphon welweschii* is more active by it is explain *in vitro* activity against the protoscolices of hydatid cyst where the protoscolices are killed after three days – post treatment in concentrations 300 µg / ml followed by 200 µg / ml that explain activity after five days - post treatment in comparison with the third concentration 100 µg/ml where revealed low activity with the others. GC- Mass spectrum of the methanol extract has explain presence of the compound Triazolo[1,5-a] pyrimidine carboxylic acid in about 13.28 % from the total composition of methanol extract of microalgae *H. welweschii*.

Key words : hydatid cyst , *H. welweschii*, methanol extract .

المستخلص :

استهدفت الدراسة الحالية اختبار فعالية المركبTriazolo[1,5-a] pyrimidine carboxylic acid ضد الكيس المائي لطفيلي المشوكة المستخلص من الطحلب الاخضر *Hapalosiphon welweschii* ضد الكيس المائي لطفيلي المشوكة الحبيبية. فقد لوحظ هناك فعالیه عاليه للمستخلص المثانولي الحاوي على المركب المستخلص اذ لوحظ قتل الرؤیسات جميعا في اليوم الثالث وبتركيز 300 µg / ml بينما لوحظت الفعالیة بعد خمسة ايام للتركيز 200 µg / ml بقتل الرؤیسات جميعا وبالمقارنه مع التركيز الثالث 100 µg/ml حيث كان اقل فعالیة بين التركيزات الاخري. وبين التحليل الكيميائي للمستخلص الميثانول لهذا الطحلب باستعمال تقنية كرومتوغرافيا الغاز طيف الكتله احتواه على نسبة عاليه من المركب Triazolo[1,5-a] pyrimidine carboxylic acid حوالى 13.28 % من المستخلص .

الكلمات المفتاحية : الكيس المائي ، الطحلب الاخضر المزرق و مستخلص الميثانول

Introduction :

Hydatid disease, hydatidosis, cystic echinococcosis, unilocular hydatid disease , *E. granulosus* Echinococcosis , and Al - akyas al-mai'yah' and 'al atash' all terms describing infections which are caused by cestodes of the genus *Echinococcus* particularly *E. granulosus* (4,10). The hydatid cyst remains a significant public health hazard in endemic areas such as Iraq , Turkey, the Middle East, South America, New Zealand , Africa , China, northern Kenya, Australia, and other sheep-

raising areas (2) . As an endemic disease, it causes social and economic losses for countries. WHO reports stated that approximately 100,000 people in the world are infected with this disease every year which is common in rural populations of underdeveloped countries because of their close association with domestic and wild animals (3).

Until recent decades, surgery was the only option for the treatment of echinococcal cysts, however, chemotherapy with benzimidazole compounds and, more recently, cyst puncture, and percutaneous aspiration, injection of chemicals, and reaspiration (PAIR) are increasingly seen to supplement or even replace surgery as the preferred treatment (4) . The undesirable side effects associated with this classical drug, as well as the development of resistance, are encouraging research into alternative synthetic or natural compounds effective for the treatment of hydatid disease . In this regard, most studies have been focused on activity of natural products from algae chlorophyta and cyanophyta , mainly due to their accessibility and use in traditional medicine . A range of pharmacological activities have also been observed with extracts of chlorophyta and cyanophyta as antibacterial , antifungal ,anticancer ,and anti-parasitic compounds (5). The treatment of the hydatid disease with plant extracts has been practiced for many centuries (2,6 , 7,8,9,10 ,11). Chlorophyta and Cyanophyta – like other algae – produce a variety of remarkable compounds collectively referred to as secondary metabolites. (5).

The aim of the following study was to estimate the activity of bioactive compound extracted from microalgae against the protoscolices of hydatid cyst for the first time .

Material and Methods :

Microalgae and methanol extract preparation :

Hapalosiphon welweschii were cultured by using Chu – 10 medium , briefly , jars of 5 liters were filled with 3 liters of liquid medium , inoculated with desired algae, and then transferred to growth chamber at 12-25 °C. Constant illumination was used at 60 $\mu\text{E}\text{m}^{-2}\text{s}^{-1}$ intensity with white fluorescent loup . Algae was harvested at the medium of stationary phase by using GFA pre weighed filter paper and centrifuge methods . Freeze – dried weighted again to reach a fixed weight of dried microalgae . The methanol extracts to be prepared ; dry mass in ratio (1: 15 g/ml) was extracted using magnetic starrier through 24 hours. The precipitates were removed by filtration and left to dry until use, and then the filtrates were concentrated at room temperature .

Parasite Materials and Protoscolices Preparation:

Fresh hydatid cysts were obtained from livers and lungs of naturally-infected sheep, which had been slaughtered at local abattoirs in Nassiriyah city, south of Iraq. They were wrapped carefully in clean plastic bags, placed in an ice box, and transported lab of cancer research unit \ College of medicine\ Thi-qar University, where protoscolices were isolated according to (17) method . the viability of protoscoleces were assessed by microscopic observation. Stained protoscoleces were considered as nonviable and the protoscoleces, which had not stained with eosin, were considered as viable ac cording to conventional. Protoscolices were counted according to method cited by (11). The viable protoscolices were counted in 1ml based on the formula :

Viability in 1 ml = number of protoscolices in (10 µl) × 100

Preparation of methanol extract and Design *In vitro* experiment :

The methanol extracts to be prepared ;dry mass in ratio (1: 15 g/ml) was extracted using magnetic starrier through 24 hours. The precipitates were removed by filtration and left to dry until use, and then the filtrates were concentrated at room temperature. The effect of bioactive chemical compounds were studied *in vitro* compared with albendazole after determination of viability of protoscolices , lethal concentrations were chose from LD₅₀ based on (11) method., *In vitro* study included Three concentration from methanol extract each of them were added alone to test tube containing 4 ml of Kreb's ringer maintain medium. The suspension of protoscolices were shaking and added to test tubes containing bioactive chemical compound in volume of 1 ml for each tube .control group was prepared with each experiment and include a test tube containing hydatid cyst fluid (Kreb's ringer mention medium + hydatid sand , 4:1) with the same viability .

- **GC-Mass spectra analysis:**

Gas chromatography - mass spectra of fraction applied for the identification and determination of the molecular weight and chemical formula and structure of the purified chemical active compounds . it was done in Bruker company , Iran and Al- Elbait university in Jordon.

Results and Discussion :

Methanol extract of *Hapalosiphon welweschii* recorded high activity at 300 µg/ml after three days - post treatment , while 100 µg/ml and 200 µg/ml has activity after five days – post treatment since the protoscolices still viable after four days – post treatment recording 6.6 and 3.3 mean of viability . further , the activity of methanol extract are seen through the first hour on the protoscolices compared with control one as explained in table (1) figure (1)

**Table (1):Viability of protoscolices treated with methanol extract of
*Hapalosiphon welweschii***

| Concentration\\ time of treatment | Mean of viability | | | | | | | | |
|------------------------------------|-------------------|-------|-------|--------|--------|--------|--------|--------|-------|
| | 1 h | 4 h | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7days |
| 100 µg/ml | 80.33 | 60.33 | 42 | 31.66 | 20 | 13.66 | 6.66 | 0 | 0 |
| 200 | 76.33 | 55 | 38.66 | 26.33 | 15.66 | 9.33 | 3.33 | 0 | 0 |
| 300 | 69.33 | 50.33 | 31.66 | 22.33 | 10 | 0 | 0 | 0 | 0 |
| Control | 95.66 | 92.66 | 90.2 | 88.33 | 80.66 | 71.66 | 69.33 | 61.66 | 58.33 |
| L.S.D. | 0.94 | | | | | | | | |
| Significant differences , P ≤ 0.05 | | | | | | | | | |



Fig (1): Pictures of treated protoscolices where :3, control group(viable protoscolices),1,2, protoscolices treated methanol extract of *Hapalosiphon welweschii*.

The GC – Mass spectrum (Fig,2) , table (2) of the methanol extract of *H. welweschii* revealed that are 22 peaks of different sizes. The results of spectrum showed that Triazolo[1,5-a] pyrimidine carboxylic acid consist 13.28 % of the total methanol extract followed by Diterpine (13.03 %) as illustrated below :

Table (2) :bioactive chemical compounds of methanol extract of *H. welweschii*

| Peak | R.T. | % of total | Compounds | M.W. |
|------|--------|------------|--|--------|
| 1 | 24.628 | 13.28 | - Triazolo[1,5-a] pyrimidine carboxylic acid | 164.12 |
| 2 | 27.673 | 13.03 | - Diterpine | 286.45 |
| 3 | 28.951 | 4.36 | - 3,7,11,15,tetramethyl- 2- hexadecan-1-ol | 296 |
| 4 | 30.362 | 2.42 | -18- Nonadecen- 1-amine | 283 |

Instrument : online
Sample Name:
Misc Info :
Vial Number: 1

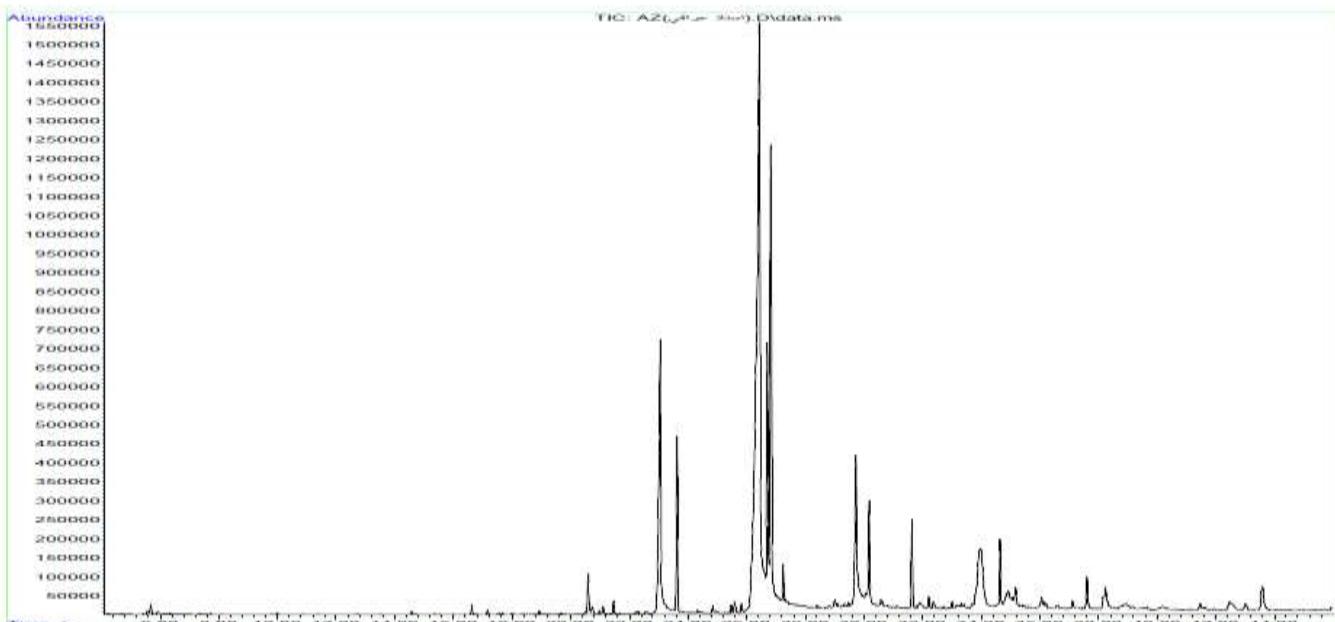


Figure (2) : GC- Mass spectrum of Methanol extract.

Natural products have been the source of therapies since the advent of traditional medicine and healing; it remains a dominant source to date. The World Health Organization (WHO) estimates that 80% of the world's inhabitants depend mainly on traditional medicine for their primary health care (1,12).

Three concentrations of the methanol extract of *H. welweschi* were used in the present study and it had an *in vitro* activity against the *hydatid cyst* and the time plays an important role in the treatment since the decreased concentration leads to increase the time of treatment. The activity of the methanol extract could be explained by the presence of the compound Triazolo[1,5-a] pyrimidine carboxylic acid. The activity of algae extracts as antibacterial and antifungal was tested previously by (3, 4, 5).

It is difficult to speculate the mechanism by which these bioactive compounds act as parasiticidal agents. In this regard (16) suggested that many bioactive chemical compounds exhibited their parasiticidal activity by virtue of their interference with the redox balance of the parasites, acting either on the respiratory chain or the cellular defenses against oxidative stress. It is also known that some bioactive compounds act by binding with the DNA of the parasite. For example, dihydroorotate dehydrogenase (DHOD), the fourth enzyme in the *de novo* pyrimidine biosynthetic pathway, is essential to parasites, including the electron acceptor capacity and cellular localization (18). In this way, it has been recently demonstrated that the methanol extracts of brown algae *Ishige okamurae*, *Fucus evanescens*, and *Pelvetia babingtonii* contain potent noncompetitive inhibitors against *Trypanosoma cruzi* DHOD (19 and 20).

Finally and based on the current study which is applied at the first time should be recommended about the use of bioactive material extracted from microalgae as antiprotoscolices against the protoscolices of hydatid cyst during surgical intervention to prevent the formation of secondary hydatid cyst from spillage of cyst content in the body of patient.

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